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Absence of Evidence for a Causal Link between Bovine Spongiform Encephalopathy Strain Variant L-BSE and Known Forms of Sporadic Creutzfeldt-Jakob Disease in Human PrP Transgenic Mice

Emilie Jaumain,[a] Isabelle Quadrio,[b,c] Laetitia Herzog,[a] Fabienne Reine,[a] Human Rezaei,[a] Olivier Andréoletti,[d] Hubert Laude,[a] Armand Perret-Liaudet,[b,c] Stéphane Haïk,[e,f] Vincent Béringue[a]

VIM, INRA, Université Paris-Saclay, Jouy-en-Josas, France[a]; Neurobiology Laboratory, Biochemistry and Molecular Biology Department, Hôpitaux de Lyon, Lyon, France[b]; University of Lyon 1, CNRS UMR5292, INSERM U1028, BioRan, Lyon, France[c]; IHAP, INRA, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France[d]; INSERM U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Université Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle Épinière, ICM, Paris, France[e]; Centre National de Référence des Agents Transmissibles Non Conventionnels, Paris, France[f]

ABSTRACT

Prions are proteinaceous pathogens responsible for subacute spongiform encephalopathies in animals and humans. The prions responsible for bovine spongiform encephalopathy (BSE) are zoonotic agents, causing variant Creutzfeldt-Jakob disease (CJD) in humans. The transfer of prions between species is limited by a species barrier, which is thought to reflect structural incompatibilities between the host cellular prion protein (PrP^{C}) and the infecting pathological PrP assemblies (PrP^{Sc}) constituting the prion. A BSE strain variant, designated L-BSE and responsible for atypical, supposedly spontaneous forms of prion diseases in aged cattle, demonstrates zoonotic potential, as evidenced by its capacity to propagate more easily than classical BSE in transgenic mice expressing human PrP^{C} and in nonhuman primates. In humanized mice, L-BSE propagates without any apparent species barrier and shares similar biochemical PrP^{Sc} signatures with the CJD subtype designated MM2-cortical, thus opening the possibility that certain CJD cases classified as sporadic may actually originate from L-type BSE cross-transmission. To address this issue, we compared the biological properties of L-BSE and those of a panel of CJD subtypes representative of the human prion strain diversity using standard strain-typing criteria in human PrP transgenic mice. We found no evidence that L-BSE causes a known form of sporadic CJD.

IMPORTANCE

Since the quasi-extinction of classical BSE, atypical BSE forms are the sole BSE variants circulating in cattle worldwide. They are observed in rare cases of old cattle, making them difficult to detect. Extrapolation of our results suggests that L-BSE may propagate in humans as an unrecognized form of CJD, and we urge both the continued utilization of precautionary measures to eliminate these agents from the human food chain and active surveillance for CJD phenotypes in the general population.

Prions are pathogens formed from abnormally folded assemblies (PrP^{Sc}) of the host-encoded prion protein PrP^{C}. Upon infection, they self-replicate by converting host PrP^{C} into PrP^{Sc} assemblies (1). Within defined host species, PrP^{C} can transconform into multiple strains differing in their PrP^{Sc} conformations and in their biological properties in tissues from reporter animals or in cell lines (2–6).

In humans, prions can form sporadically and induce Creutzfeldt-Jakob disease (CJD), mainly in elderly people. The worldwide incidence of sporadic CJD (sCJD) ranges from one to two cases per million people per year (7). Evaluation of both the PrP^{Sc} electrophoretic pattern after proteinase K treatment and the methionine/valine polymorphism at codon 129 of the gene encoding PrP allows the definition of multiple sCJD molecular subtypes, which exhibit specific clinical and neuropathological features (8–10). The most common form of sCJD is associated with the presence of type 1 (T1) protease-resistant PrP^{Sc} (PrP^{res}) and methionine homozygosity (MM) at codon 129. Rare forms of MM sporadic CJD with a type 2 (T2) PrP^{res} and further subclassified as cortical and thalamic variants (9, 11, 12) have also been reported.

The implementation of active surveillance programs for the presence of PrP^{res} in the nervous tissues of livestock has led to the recognition of two novel bovine prion strain types, both distinct from the prion responsible for the bovine spongiform encephalopathy (BSE) epidemics. These types are designated L-BSE and H-BSE and refer to the brain PrP^{res} electrophoretic pattern in comparison with "classical" BSE (C-BSE). Both types have been detected worldwide in aged animals, with a low prevalence consistent with the existence of sporadic forms of prion diseases in cattle (13).

The transfer of prions between species is governed by the possibility of cross-interactions between the host PrP^{C} and the invading PrP^{Sc} type (4, 14). Mice transgenic for mammalian PrP^{C} (on a mouse PrP^{C}-ablated background) are thus highly relevant models to use in the experimental investigation of the ability of prions to

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Address correspondence to Vincent Béringue, vincent.beringue@inra.fr.

TABLE 1 Transmission of atypical L-BSE and sporadic CJD prions to transgenic mice expressing human prion protein (Met_{129})

Isolate[a]	Origin (identification no.)	Survival time (days [mean ± SEM])[b]			
		1st passage	2nd passage	3rd passage	4th passage
L-type BSE	Italy (1088)	*607 ± 23 (9/9)*	*653 ± 13 (11/11)*	620 ± 11 (8/8)	622 ± 10 (8/8)
	France (7)	*574 ± 35 (7/7)*	624 ± 15 (12/12)	624 ± 14 (8/8)	617 ± 21 (7/7)
	France (10)	*703 ± 19 (8/8)*			
	France (11)	*647 ± 26 (9/9)*			
MM1-sCJD	UK (NHBX0/0002)	*177 ± 1 (8/8)*	*153 ± 3 (4/4)*		
	UK (NHBX0/0001)	*153 ± 3 (4/4)*	*157 ± 2 (6/6)*	156 ± 2 (6/6)	159 ± 2 (6/6)
	France (2)	*159 ± 3 (6/6)*	*161 ± 2 (6/6)*		
	France (3)	*162 ± 3 (6/6)*			
	France (4)	*167 ± 2 (6/6)*			
MM2-sCJD (cortical)	France (1)	*278 ± 6 (6/6)*	*274 ± 4 (8/8)*	*280 ± 4 (12/12)*	*268 ± 4 (8/8)*
MV1-sCJD	France (1)	162 ± 2 (6/6)	153 ± 3 (4/4)		
	France (2)	159 ± 2 (5/5)			
	France (3)	156 ± 2 (5/5)			
MV2-sCJD	UK (NHBXY0/0004)	486 ± 22 (8/8)	431 ± 29 (4/4)	418 ± 12 (4/4)	414 ± 3 (7/7)
	France (1)	474 ± 5 (6/6)	448 ± 8 (12/12)	413 ± 16 (6/6)	
	France (2)	504 ± 20 (7/7)	431 ± 24 (5/5)	453 ± 5 (9/9)	
	France (3)	450 ± 2 (6/6)	405 ± 10 (7/7)	435 ± 8 (7/7)	414 ± 6 (6/6)
VV1-sCJD	France (1)	597 ± 31 (5/5)	528 ± 5 (5/5)	461 ± 6 (8/8)	435 ± 10 (8/8)
VV2-sCJD	France (1)	566 ± 21 (8/8)	433 ± 18 (6/6)	427 ± 11 (4/4)	447 ± 6 (10/10)
	France (2)	500 ± 57 (6/6)	459 ± 13 (5/5)	451 ± 4 (5/5)	461 ± 18 (6/6)
	France (3)	504 ± 22 (8/8)	446 ± 19 (6/6)	449 ± 5 (3/3)	455 ± 7 (8/8)

[a] Sporadic CJD (sCJD) cases were classified according to the genotype at codon 129 of *PRNP* (MM, methionine homozygote; VV, valine homozygote; MV, methionine-valine heterozygote) and PrP^{res} type by Western blotting (1, type 1 PrP^{res}; 2, type 2 PrP^{res}).

[b] Survival was determined after intracerebral inoculation with 2 mg of brain tissue equivalent. The values in parentheses represent the number of affected (clinical signs and PrP^{res}-positive) animals/number of inoculated animals. Data in italics are from references 16, 24, and 32.

propagate in foreign species. In particular, mouse models expressing human PrP are helpful for gauging the zoonotic potential of animal prions (6, 15). Similar to C-BSE prions, H- and L-type prions can propagate in nonbovine species and in transgenic mouse models expressing nonbovine PrPs (reviewed in reference 2). L-BSE prions replicated faster than C-BSE prions in the brains of transgenic mice expressing human PrP with methionine at codon 129 (Met_{129}; *tg650* line [16]), as indicated by the kinetics of PrP^{res} appearance (17). Further investigation of the virulence of this agent revealed that L-BSE prions, unlike C-BSE prions, could propagate in these mice with no apparent barrier (6, 15, 16). L-type BSE was also transmitted to two other genetically different lines of humanized mice (Met_{129}) (18, 19) and to nonhuman primates (20, 21). In these animal models, the L-type PrP^{res} electrophoretic pattern in the brain intriguingly resembled that found in the brains of sCJD patients with T2 PrP^{res} before (18) or after passage in these mice (17), therefore raising the possibility that both agents were causally linked. Recently, the serial transmission of multiple sheep scrapie isolates to mice expressing human PrP led to the isolation of prions phenotypically identical to those causing sCJD, further strengthening the view that animal prions may cause human prion diseases (22).

Here, we used transgenic mice expressing human PrP to compare the phenotypic traits of L-BSE isolates with those from a series of human sCJD cases with various genotypes at codon 129 and various electrophoretic signatures, which are representative of the human prion strain diversity (23, 24).

MATERIALS AND METHODS

Ethics statement. All animal experiments were approved by the INRA Local Ethics Committee (Comethea; permit number 12/034). Human tissues samples were selected from the French National Center of Reference for Unconventional Transmissible Agents and from the National Institute for Biological Standards and Control (NIBSC) CJD Resource Centre. For each case, an informed consent for genetic analysis of the PrP gene (*PRNP*) to obtain the genotype at codon 129 and to exclude the presence of pathogenic mutations was obtained from the patient's relatives at the time of diagnosis. The patient's relatives also gave written informed consent for autopsy and research using postmortem tissues in accordance with French regulations (L.1232-1 to L.1232-3, Code de la Santé Publique).

Human PrP mouse line. The human PrP *tg650* line has been described previously (16). This line is homozygous and exhibits approximately 6-fold overexpression of human PrP^{C} (Met_{129} allele) in the brain. The bovine PrP *tg110* line has been described previously (25).

Prion isolates and transmission. sCJD cases were classified according to the genotype at codon 129 of *PRNP* (MM, methionine homozygote; VV, valine homozygote; MV, methionine-valine heterozygote), and the PrP^{res} type was determined by Western blotting (1, type 1 PrP^{res}; 2, type 2 PrP^{res}). Frontal cortex, striatum, or cerebellum extracts were used as sCJD material. L-BSE isolates were previously described (17). To avoid any cross-contamination, a strict protocol based on the use of disposable equipment and preparation of all inocula in a class II microbiological cabinet was followed. Tissue extracts were prepared as a 10% (wt/vol) homogenate in 5% (wt/vol) glucose for inoculation into *tg650* mice. Twenty microliters was inoculated intracerebrally into the right hemisphere of groups of individually identified 6- to 8-week-old *tg650* mice at

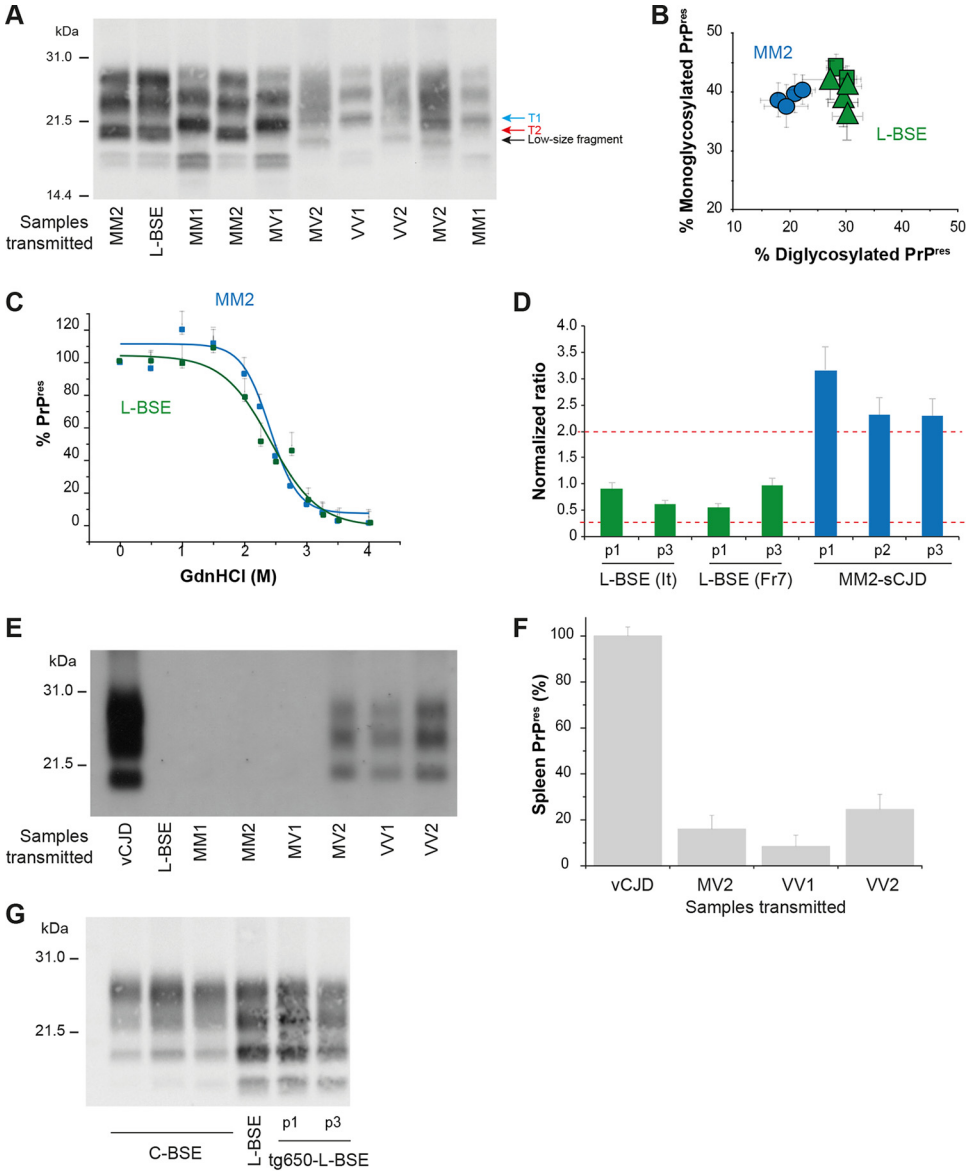

FIG 1 Glyco-pattern and conformational stability of PrP^{res} from humanized PrP mice infected with L-BSE and sCJD subtypes. (A) Immunoblot analysis of PrP^{res} in the brains of human transgenic mice (*tg650*) infected with L-BSE or sCJD subtypes (4th passage, except for MV1, which was analyzed at 2nd passage). Immunoblots were probed with the Sha31 anti-PrP monoclonal antibody. Blue and red arrows denote unglycosylated PrP^{res} bands migrating at 21 kDa (T1) and 19 kDa (T2), respectively. The black arrow indicates the additional presence of low-molecular-mass PrP^{res} fragments, which were observable specifically after infection with MV2 and VV2 sCJD sources. Molecular masses of protein standards are indicated in kilodaltons. (B) Ratio of diglycosylated to monoglycosylated PrP^{res} species in the brains of *tg650* mice following serial transmission (up to 4 passages) of cortical MM2-sCJD (blue circle) and L-BSE (green triangles, Italian BASE isolate; green squares, Fr7 isolate) prions (data plotted as the means ± standard errors of the means; $n = 6$ mice analyzed at each passage). (C) Relative resistance of L-BSE and MM2-sCJD prions to guanidinium chloride (GdnHCl)-induced denaturation. Analysis was performed at the 3rd passage in *tg650* mice by immunoblotting. The measures shown are the means ± standard errors of the means from $n = 3$ independent experiments run in duplicate (3 mouse brains analyzed per prion). (D) Relative resistance of L-BSE and MM2-sCJD prions to proteinase K. Analysis was performed over passaging (p) in *tg650* mice. PrP^{Sc} in brain homogenates from terminally ill mice was purified in two buffers containing distinct proteinase K concentrations and various detergent conditions and then analyzed using a commercial enzyme-linked immunosorbent assay kit (TeSeE CJD; Bio-Rad) (10). After normalization of results, samples could be split into the following categories of resistance to proteinase K based on the ratio of PrP^{res} species: resistant, <0.3 (bottom red dotted line), intermediate, >0.3 and <1; sensitive, >1 and <2; and highly sensitive, >2 (top red dotted line). The measures shown are the means ± standard errors of the means from three independent experiments run in duplicate. (E and F) Immunoblot analysis and relative accumulation of PrP^{res} in the spleens of *tg650* mice infected with L-BSE prions or sCJD subtypes. The level detected after inoculation with the lymphotropic variant CJD (vCJD) prion is shown for comparison (100% in F). Immunoblots were probed with the Sha31 anti-PrP monoclonal antibody. Quantification was performed by using 10 to 14 spleens at the 3rd or 4th passage (means ± standard errors of the means). (G) Back-passage of humanized L-BSE prions to bovine PrP transgenic mice. *tg650*-derived L-BSE prions (tg650-L-BSE) at the first (p1) and third (p3) passages were inoculated intracerebrally into bovine PrP transgenic mice (*tg110* line). The brains of the diseased mice were analyzed for PrP^{res} content using Western blotting. The glyco-pattern of L-BSE and C-BSE prions in *tg110* mice is shown for comparison (Sha31 antibody).

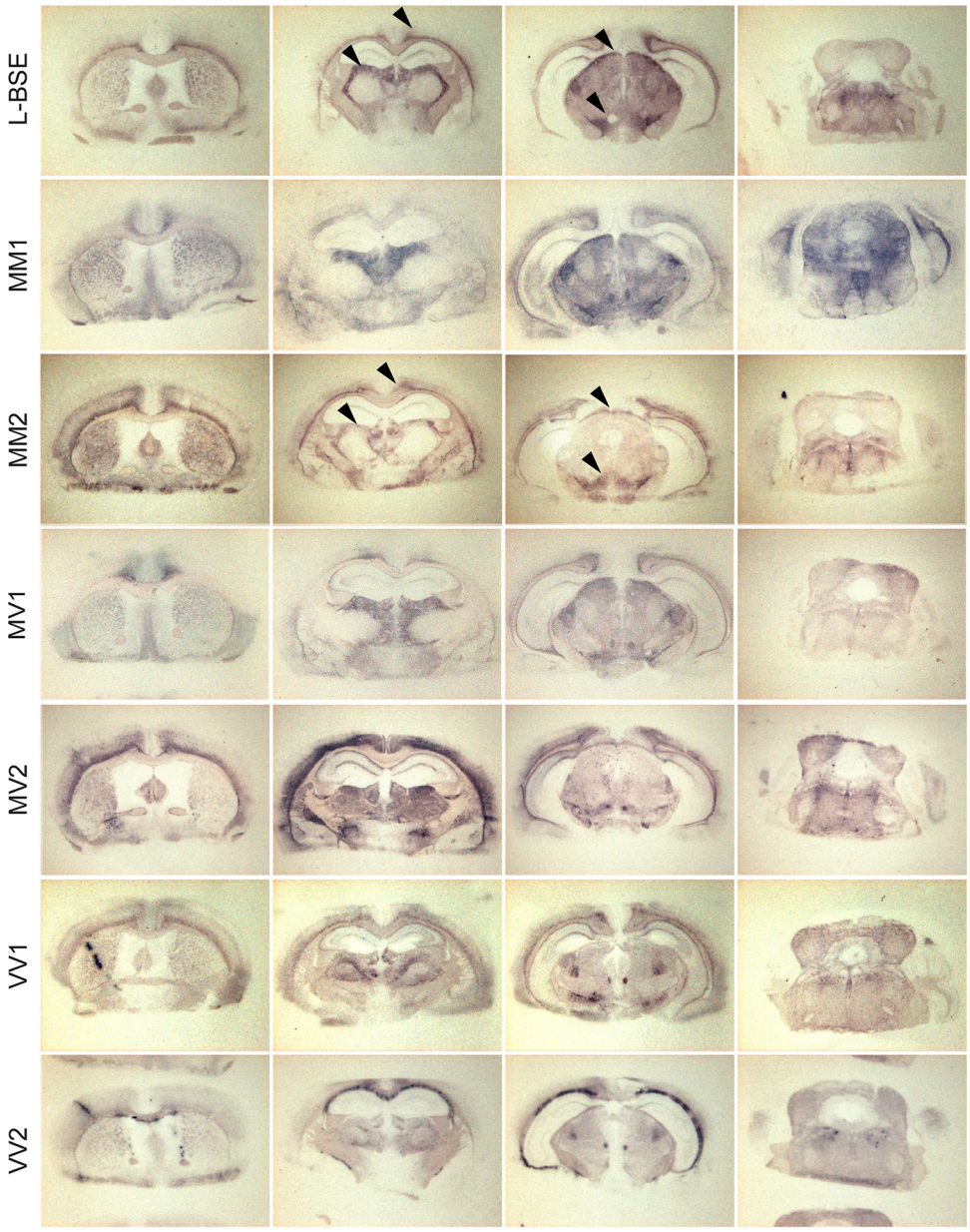

FIG 2 Neuroanatomical distribution of PrP^{res} in human PrP transgenic mice challenged with L-type BSE prions or human CJD subtypes. Representative histoblots in four antero-posterior sections showing the deposition of disease-specific PrP^{res} deposits in the brains of *tg650* mice infected with L-BSE prions or sCJD prions, as indicated. Arrowheads point to similar PrP^{res} deposition patterns after challenge with L-BSE and MM2-sCJD prions. Note the difference in the appearance of the PrP^{res} deposits of the VV2 and MV2 sCJD subtypes. Analysis was performed at the 3rd passage. Histoblots were probed with 3F4 anti-PrP monoclonal antibody.

the level of the parietal cortex. The first mouse to succumb to the disease was used for subpassaging. The brain of this mouse was collected with separate, disposable tools, homogenized at 20% (wt/vol) in 5% glucose, and reinoculated intracerebrally at 10% (wt/vol). For the back-passage of *tg650* L-BSE-derived prions to *tg110* mice, 20 μl of 10% (wt/vol) brain homogenate from mice at the terminal stage of disease was inoculated via the intracerebral route.

Immunoblot analyses. PrP^{res} was extracted from 20% (wt/vol) tissue homogenates with a Bio-Rad TeSeE detection kit as previously described (17). Samples were run on 12% bis-Tris precast gels, electrotransferred onto nitrocellulose membranes, and probed with the Sha31 anti-PrP monoclonal antibody (human PrP epitope at residues 145 to 152 [26]). Immunoreactivity was visualized by chemiluminescence. The size and relative amounts of the PrP^{res} glycoforms were determined using GeneTools software after the acquisition of chemiluminescent signals with a GeneGnome digital imager (Syngene, Cambridge, United Kingdom).

Conformation stability assays. Brain homogenates were brought to the desired concentration in guanidine HCl, incubated for 1 h at 20°C with gentle agitation, and then diluted to reach a 0.5 M guanidine HCl concentration. The samples were then digested with proteinase K (PK; 50 μg/ml final concentration), methanol precipitated, and analyzed by immunoblotting as described previously (27).

Differential proteinase K digestion. Strain-specific conformational variations in PrP^{Sc} species from the brains of transgenic mice were studied

by applying two different PK digestions under different detergent conditions, as comprehensively described previously (10).

Histoblot analyses. Brains were rapidly removed from euthanized mice and frozen on dry ice. Cryosections were cut at 8 to 10 μm, transferred onto Superfrost slides, and maintained at −20°C until use. Histoblot analyses were performed as previously described (6), using the 3F4 anti-PrP antibody (human PrP epitope at residues 107 to 112 [28]). The sections presented are representative of the results from three brain samples.

Thioflavin-S binding. After methanol fixation, brain sections were incubated with 0.01% thioflavin-S for 1 h as previously described (16). The sections were then incubated with the nuclear marker 4′,6-diamidino-2-phenylindole (Sigma) and mounted in Fluoromount-G (Interchim) before image acquisition with an inverted fluorescence microscope (Zeiss Axiovert) and analysis with Zeiss Axiovision software.

RESULTS

Human PrP *tg650* mice (Met_{129} allele) (16) were challenged with prions from L-BSE isolates and prions from 17 cases of sporadic CJD from France and the United Kingdom. These prions were passaged iteratively (via the intracerebral route) three to four times in *tg650* mice. Disease incidence and potential reduction of incubation duration were used to estimate the magnitude of the species/transmission barrier. Prions that propagated in the brain and spleen tissue of the infected *tg650* mice were characterized using standard biochemical and immunohistochemical criteria used to distinguish between prion strains (29–31).

Comparison of the mean times to disease onset over three to four passages in *tg650* mice showed no overlap between L-BSE and any of the sCJD subtypes transmitted (Table 1). The L-BSE incubation time (>600 days) was found to be significantly longer than that observed with all the sCJD subtypes (<500 days; $P < 0.05$, Kruskal-Wallis test). According to the mean incubation times, the sCJD subtypes were segregated into three groups: MM1/MV1, which induced the fastest disease in mice, i.e., within 160 days; MM2, which induced disease in ~270 days; and MV2/VV1/VV2, which induced disease between 410 and 460 days. At the fourth passage, the mean incubation times of mice inoculated with the MV2 and VV2 subtypes were not significantly different ($P = 0.47$, Kruskal-Wallis test).

The PrP^{res} molecular glycoprofile in *tg650* diseased mouse brains was relatively uniform after the serial passaging of L-BSE prions and sCJD prions. The L-type profile was characterized, as in cows, by a 19-kDa band for unglycosylated PrP^{res} and by a monoglycosylated dominant PrP^{res} pattern (Fig. 1A and B) (32). This profile closely resembled that observed only after the transmission of MM2-sCJD to *tg650* mice (Fig. 1A and B). The same observation was made after transmission to nonhuman primates (20). The other CJD cases produced distinct signatures (Fig. 1A) (24) characterized by an ~21-kDa band for unglycosylated PrP^{res} (MM1, MV1, MV2, VV1, and VV2) and by the presence of lower-molecular-mass fragments (MV2 and VV2). The predominant type 2 signature in the patient brain was thus lost from all isolates, except the MM2-sCJD isolate, upon transmission to mice expressing the Met_{129} allele of human PrP.

We next examined whether *tg650*-passaged MM2-sCJD and L-type prions could, however, be distinguished biochemically. Comparison of the conformational stabilities of the associated PrP^{res} molecules after exposure to increasing concentrations of guanidinium chloride (27) failed to identify significant differences (Fig. 1C). However, their relative resistance to differential proteinase K digestion, an alternative tool for CJD molecular typing (10), revealed differences. *tg650*-derived L-type PrP^{res} was classified as intermediate with regard to proteinase K resistance, whereas MM2-sCJD PrP^{res} remained highly sensitive over three passages (Fig. 1D).

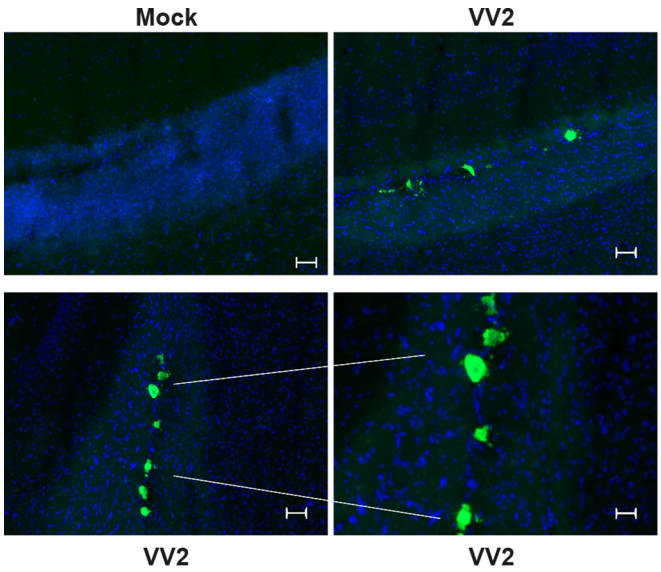

FIG 3 The presence of amyloid plaques in human PrP mice on serial passage of VV2-sCJD prions. Coronal brain sections from *tg650* mice infected with VV2-sCJD-derived prions were stained with the nuclear marker 4′,6-diamidino-2-phenylindole (blue) and thioflavin-S s (green). Analysis was performed at the level of the external capsule (top) or the cingulum (bottom). Mock-inoculated *tg650* mice were used as a negative control. Scale bar, 50 μm.

The panel of sCJD and L-BSE prions exhibited various degrees of lymphotropism for *tg650* mouse spleen tissue, based on PrP^{res} detection. L-type PrP^{res} was not detected in the spleens of infected *tg650* mice, regardless of the number of passages, suggesting that this agent was poorly lymphotropic, as were MM1- and MM2-sCJD (16, 24). The other CJD subtypes exhibited low but consistent levels of PrP^{res} in *tg650* mouse spleens (Fig. 1E). The accumulation observed ranged from 4-fold-lower (VV2) to 10-fold-lower (VV1) than that observed with variant CJD (vCJD) (Fig. 1G) (16), suggesting a poorer, albeit significant, lymphotropism.

The strain-specified (2) neuroanatomical distribution of PrP^{res} deposits in the brain was studied by histoblotting. L-BSE exhibited a PrP^{res} deposition pattern in the *tg650* mouse brain that was superimposable only on MM2-sCJD prions (Fig. 2). PrP^{res} deposition was prominent in the dorsal and habenular thalamic nuclei, in the optic tract, in the cingulum, in the external capsule, in the lateral hypothalamic area, and in the trigeminal nuclei of *tg650* mouse brains (Fig. 2) (24). Variations in PrP^{res} distribution patterns and in the nature of the deposits among the sCJD types allowed further segregation into five distinct strain types: MM1/MV1, MM2, MV2, VV1, and VV2. In particular, there were granular, plaque-like deposits specifically present in the corpus callosum of VV2-inoculated mice, whereas MV2 deposits were thinner and mostly located in the thalamus and in the cortical areas (Fig. 2). The VV2 deposits were thioflavin-S positive, indicating an amyloid fibril organization (Fig. 3).

Finally, we examined whether L-BSE prions "breed true" in *tg650* mice. *tg650*-derived L-BSE agents at the first to third passage

TABLE 2 Back-passage of *tg650*-derived L-BSE prions into bovine PrP mice

Inoculum	Passage no.	Incubation time (days [mean ± SEM])
tg650-L-type	1	233 ± 14 (6/6)[a]
	2	210 ± 5 (6/6)
	3	214 ± 2 (6/6)
Bovine L-type	1	245 ± 15 (8/8)

[a] Values in parentheses represent the number of mice with neurological disease and positive for PrP^{res} in the brain by immunoblotting/number of inoculated *tg110* mice.

were transmitted back into bovine PrP mice (25) via the intracerebral route. As shown in Table 2, *tg650*-derived L-BSE prions were as pathogenic as parental L-BSE prions in these mice. Based on the PrP^{res} glyco-pattern in the brains of the diseased mice, the strain type reisolated was fully consistent with L-BSE prions (Fig. 1G). Together, these data indicate that L-BSE prions were propagated in human PrP mice without an apparent transmission barrier.

DISCUSSION

We further demonstrated in this study that atypical L-BSE prions propagate with no significant barrier in human PrP transgenic mice (Met_{129}), as shown by the full disease incidence, the absence of a drastic reduction in incubation time over four passages, the conservation of the L-type PrP^{res} electrophoretic pattern, and the immediate reisolation of L-BSE prions in bovine PrP transgenic mice from the first back-passage onwards. Although gene-targeted transgenic mice expressing physiological levels of human PrP did not show any clinical disease on primary challenge with L-BSE prions (33), they were later found to harbor remnant or low levels of infectivity in their brains (34), thus suggesting that the disease developed at a slow pace. Nonhuman primates, which can live longer, were found to succumb to L-BSE more rapidly than C-BSE (20, 21). Together, these data indicate that L-BSE prions have a clear zoonotic potential.

We found no evidence that L-BSE prions cause a known form of sporadic CJD. Although *tg650*-derived L-BSE prions share similar strain properties with cortical MM2-sCJD prions in terms of PrP^{res} signature, resistance to guanidinium chloride treatment, limited tropism for the lymphoid tissue, and neuroanatomical PrP^{res} deposition, the 2-fold difference in disease progression over at least four passages and the differential resistance to proteinase K digestion suggest that there is no etiological link between L-BSE and cortical MM2-sCJD prions. This absence of an etiological link was further substantiated by the divergent transmission properties of the two agents in ovine PrP transgenic mice (17, 24). The extreme rareness of the cortical sCJD MM2 subtype precluded a comparison with a larger panel of cases to definitively exclude any etiological link.

Our study confirmed the existence of diverse strains of prions associated with sCJD. We identified four distinct groups, MM1/MV1, MM2-cortical, MV2/VV2, and VV1, as previously inferred from the transmission or absence of transmission of these subtypes in gene-targeted transgenic mice expressing human PrP (23, 35). The MV2 and VV2 subtypes, however, showed dissimilar neuroanatomical distributions of PrP^{res} deposits. Further studies that include transmission to other human PrP genotypes are needed to confirm whether MV2 and VV2 prions are truly differentiable with regard to their biological properties.

The *tg650* mouse model allows the gauging of prion ability to replicate in the lymphoid tissue (6, 16, 36). We showed here that MV2, VV2, and VV1 sCJD prions replicated in *tg650* mouse spleens, albeit at a lower rate than vCJD, as determined by PrP^{res} accumulation levels in this tissue. This result suggests that the commonly shared view that sCJD prion replication is primarily confined to the central nervous system (37–39) is subtype dependent. Refined analysis of spleen tissue from sCJD-affected patients demonstrated the presence of PrP^{res} in all subtypes (40). The presence of PrP^{res} appeared to correlate with longer duration of disease, as we found here. Systematic analysis of clinical sCJD subtypes for the presence of infectivity or seeding activity (41–43) in central and extraneural tissues (44, 45) is needed to assess the risk of sCJD iatrogenic spread after surgical procedures in preclinical patients.

Careful extrapolation of our data raises the possibility that an unrecognized human prion strain type may emerge from the accidental transfer of L-BSE prions to humans. Together with the risk that L-BSE prions will propagate with better efficacy in humans than C-BSE prions (19–21, 32), these data highlight the need for continued long-term utilization of precautionary measures to diagnose (46) and prevent these agents from entering the human food chain and for the maintenance of an active surveillance of prion strains within the CJD population.

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We declare that we have no competing interests.

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